

What is claimed is:

1. A barley plant, or a part thereof, comprising less than 5% of the LOX-1 activity of a wild-type barley plant.
- 5 2. The barley plant, or a part thereof, according to claim 1, wherein said part of said barley plant is kernel(s).
3. The barley plant, or a part thereof, according to claim 1, wherein the embryos of
10 said plant comprise less than 5% of the LOX-1 activity of the embryos of a wild-type barley plant.
4. The barley plant, or a part thereof, according to any of claims 1 to 3, wherein said
15 plant or part thereof comprises less than 1% LOX-1 protein compared to a wild-type barley plant.
5. The barley plant, or a part thereof, according to any of claims 1 to 4, wherein said
plant is produced by a method comprising the steps of; or said plant is progeny of a
plant produced by a method comprising the steps of:
20 (i) determining the LOX-1 activity in wild-type barley kernels or parts
 thereof; and
 (ii) mutagenizing barley plants, and/or barley kernels, and/or barley
 embryos, and/or barley cells and/or barley tissue, thereby obtaining
 barley of generation M₀; and
25 (iii) breeding said mutagenized barley plants, kernels, cells, tissue and/or
 embryos, for at least 2 generations, thereby obtaining barley plants of
 generation M_x, wherein x is an integer ≥ 2 ; and
 (iv) obtaining kernels or parts thereof from said barley plants of
 generation M_x; and
30 (v) determining the LOX-1 activity in said kernels or parts thereof; and
 (vi) selecting plants wherein the LOX-1 activity of the mutagenized
 kernels or parts thereof is less than 5% than the LOX-1 activity of the
 wild-type kernels or part thereof.

6. The barley plant, or a part thereof, according to any of claims 1 to 5, wherein the gene encoding LOX-1 of said plant comprises a premature nonsense codon.

5 7. The barley plant, or a part thereof, according to claim 6, wherein the gene encoding LOX-1 of said plant comprises a nonsense codon, said codon corresponding to base no.s 3572–3574 of SEQ ID NO: 2.

10 8. The barley plant, or a part thereof, according to claim 6, wherein said plant is selected from the group consisting of plants designated D112 having American Type Culture Collection (ATCC) deposit accession No. PTA-5487, and progeny plants thereof.

15 9. The barley plant, or a part thereof, according to any of claims 1 to 5, wherein the gene encoding LOX-1 of said plant comprises at least one mutation within a splice site.

20 10. The barley plant, or a part thereof, according to claim 9, wherein the gene encoding LOX-1 of said plant comprises a splice site mutation, said mutation corresponding to base no. 2311 of SEQ ID NO: 6.

11. The barley plant, or a part thereof, according to claim 9, wherein said plant is selected from the group consisting of plants designated A618 having ATCC deposit accession No. PTA-5584, and progeny plants thereof.

25 12. The barley plant, or a part thereof, according to any of claims 1 to 11, wherein said plant is characterized by:

- 30 (i) having enhanced disease resistance; or
(ii) having reduced potential for the production of mycotoxins; or
(iii) comprising regenerable cells for use in tissue culture; or
(iv) any combination of the traits of (i) to (iii).

13. The barley plant, or a part thereof, according to claim 12, further characterized by the presence of a gene encoding LOX-1, wherein said gene comprises:

- 35 (i) a premature nonsense codon; or
(ii) a splice site mutation.

14. The barley plant according to claim 13, further characterized by the presence of a gene encoding LOX-1, said gene comprising:

- 5 (i) a nonsense codon corresponding to base no.s 3572–3574 of
SEQ ID NO: 2; or
- (ii) a splice site mutation corresponding to base no. 2311 of
SEQ ID NO: 6.

10 15. A composition comprising the barley plant or part thereof according to any of claims 1 to 14.

16. A malt composition comprising a processed barley plant or part thereof, wherein said barley plant is the barley plant according to any of claims 1 to 14.

15 17. The malt composition according to claim 16, wherein said part of said barley plant is kernel(s).

20 18. A wort composition prepared using the barley plant or part thereof according to any of claims 1 to 14 or using a malt composition prepared from said barley plant or part thereof or mixtures thereof.

19. The wort composition according to claim 18, wherein said part of said plant is kernel(s).

25 20. The wort composition according to claim 18, wherein said malt composition is a malt compositions according to any of claims 16 and 17.

30 21. The wort composition according to any of claims 18 to 20, wherein said composition is prepared using an enzyme composition or an enzyme mixture composition.

22. A composition prepared from a mixture of (i) a composition comprising a barley plant or a part thereof according to any of claims 1 to 14, and (ii) a malt composition according to any of claims 16 and 17.

35

23. A wort composition or a beverage prepared from the composition of claim 22.

24. A beverage having stable organoleptic qualities, wherein said beverage is obtained by manufacturing the barley plant or part thereof according to any of claims 1 to 14.

25. The beverage according to claim 24, wherein said beverage is beer.

26. The beverage according to claim 24, wherein said beverage is prepared using malt prepared from kernels of said barley plant.

27. The beverage according to any of claims 24 to 26, wherein said beverage is prepared from a wort composition prepared from a barley plant or part thereof, or from a malt composition prepared from said barley plant or part thereof, wherein said barley plant comprises less than 5% of the LOX-1 activity of a wild-type barley plant.

28. The beverage according to claim 24, wherein said beverage is prepared from unmalted barley plants or parts thereof.

29. The beverage according to any of claims 24 to 28, wherein said beverage is a non-fermented beverage

30. The beverage according to any of claims 24 to 29, wherein said barley plant, or parts thereof, comprise a gene encoding LOX-1, said gene comprising:

- (i) a nonsense codon; or
- (ii) a splice site mutation.

31. The beverage according to claim 30, wherein the gene encoding LOX-1 comprises:

- (i) a nonsense codon, said codon corresponding to base no.s 3572–3574 of SEQ ID NO: 2; or
- (ii) a splice site mutation, said mutation corresponding to base no. 2311 of SEQ ID NO: 6.

32. A beverage having stable organoleptic qualities, wherein said beverage is manufactured by using a barley plant, wherein the ratio of 9,12,13-trihydroxyoctadecenoic acid to 9,10,13-trihydroxyoctadecenoic acid within said beverage is at the most 1.8.

5

33. The beverage according to claim 32, wherein said beverage is prepared by fermentation of a barley plant, or parts thereof, or extracts thereof, and wherein said barley plant comprises less than 5% of the LOX-1 activity of a wild-type barley plant.

10

34. The beverage according to any of claims 32 to 33, wherein said beverage is beer.

35. A beverage having stable organoleptic qualities, wherein said beverage is manufactured by using a barley plant, and wherein said beverage comprises at the most 0.05 ppb free *trans*-2-nonenal (T2N) after incubation at 37°C for 4 weeks, in the presence of in the range of 4 to 6 ppm sulfite.

15

36. The beverage according to claim 35, wherein the beverage is manufactured by fermentation of a barley plant, or parts thereof, or extracts thereof, and wherein said barley plant comprises less than 5% of the LOX-1 activity of a wild-type barley plant.

20

37. The beverage according to any of claims 35 to 36, wherein the ratio of 9,12,13-trihydroxyoctadecenoic acid to 9,10,13-trihydroxyoctadecenoic acid within said beverage is at the most 1.8.

25

38. The beverage according to any of claims 35 to 37, wherein said beverage is beer.

39. A plant product produced from the barley plant, or a part thereof, according to any of claims 1 to 14.

30

40. The plant product according to claim 39, wherein said plant product is a beverage.

35

41. A method of producing:

- (i) a food composition; or
- (ii) a feed composition; or
- (iii) a fragrance raw material composition; or
- (iv) any combination of (i) to (iii);

5 using a barley plant or part thereof according to any of claims 1 to 14.

42. A food composition, a feed composition, or a fragrance raw material composition comprising the barley plant or part thereof according to any of claims 1 to 14.

10 43. A method for expressing a recombinant protein in barley to obtain a barley plant according to any of claims 1 to 14, wherein said method comprises stably transforming said plant with a nucleic acid sequence comprising, as operably linked components, a promoter expressible in barley plants or parts thereof, a DNA sequence encoding said recombinant protein, and a transcriptional termination
15 region.

44. A method of modulating levels of a protein in barley to obtain a barley plant according to any of claims 1 to 14, the method comprising stably transforming said plant with a nucleic acid sequence comprising, as operably linked components, a
20 promoter expressible in barley plants, a DNA sequence, and a transcriptional termination region, wherein expression of said DNA sequence reduces the expression of a gene encoding said protein by:

- (i) antisense silencing; or
- (ii) co-suppression silencing; or
- 25 (iii) RNA interference.

45. A method of preparing the barley plant according to any of claims 1 to 14, the method comprising stably transforming a barley plant with a nucleic acid sequence comprising, as operably linked components, a promoter expressible in barley
30 plants, a DNA sequence, and a transcriptional termination region, wherein expression of said DNA sequence reduces the expression of the gene encoding LOX-1 by:

- (i) antisense silencing; or
- (ii) co-suppression silencing; or
- 35 (iii) RNA interference.

46. A method of producing a beverage having stable organoleptic qualities, said method comprising the steps of:

- 5 (i) preparing a composition comprising a barley plant or parts thereof according to claim 1;
- (ii) processing the composition of (i) into a beverage;
- thereby obtaining a beverage with stable organoleptic qualities.

10 47. The method according to claim 46, wherein step (i) comprises preparing a malt composition from kernels of said barley plant or part thereof.

48. The method according to any of claims 46 and 47, wherein the method further comprises incubation with a LOX inhibitor.

15 49. The method according to claim 46, wherein processing the composition into a beverages comprises a mashing step.

50. The method according to claim 46, wherein a LOX inhibitor is added during said mashing step.

20

51. A method of producing a malt composition with low or no LOX-1 activity, said method comprising the steps of:

- (i) providing kernels according to claim 2;
- (ii) steeping said kernels;
- 25 (iii) germinating the steeped kernels under predetermined conditions;
- (iv) treating germinated kernels with heat;
- thereby producing a malt composition with no or low LOX-1 activity.

30 52. A method of preparing a barley plant comprising less than 5% of the LOX-1 activity of a wild-type barley plant comprising the steps of:

- (i) determining the LOX-1 activity in wild-type barley kernels or parts thereof; and
- (ii) mutagenizing barley plants and/or barley kernels and/or barley embryos and/or barley cells and/or barley tissue thereby obtaining
- 35 generation M0 barley; and

1. (iii) breeding said mutagenized barley plants, kernels, cells, tissue and/or embryos for at least 2 generations, thereby obtaining generation Mx barley plants, wherein x is an integer ≥ 2 ; and
- (iv) obtaining kernels or parts thereof from said Mx barley plants; and
- 5 (v) determining the LOX-1 activity in said kernels or parts thereof; and
- (vi) selecting plants wherein the LOX-1 activity of the mutagenized kernels or parts thereof is less than 5% than the LOX-1 activity of the wild-type kernels or part thereof;
- thereby obtaining a barley plant comprising less than 5% of the LOX-1 activity
- 10 of a wild-type barley plant.

53. A method of preparing a barley plant comprising less than 5% of the LOX-1 activity of a wild-type barley plant, wherein the method comprises the steps of:

- (i) mutagenizing barley plants, and/or barley kernels and/or barley embryos; and
- 15 (ii) optionally breeding said mutagenized barley plant/barley kernel/barley embryo; and
- (iii) determining the presence or absence of a mutation in the barley gene encoding LOX-1, said mutation leading to a gene encoding a polypeptide form of LOX-1 comprising less than 700 contiguous amino acids of the sequence set forth in SEQ ID NO: 3; and
- 20 (iv) selecting plants carrying the mutation provided in (ii).

54. A method of reducing the activity of barley LOX, said method comprising the steps of

25

- (i) providing a barley plant or part thereof or a plant product prepared from barley,
- (ii) providing a LOX inhibitor, wherein said LOX inhibitor is a gallate
- (iii) incubating said barley plant or part thereof or plant product prepared
- 30 from barley with said LOX inhibitor, thereby reducing the activity of barley LOX (preferably LOX-1).

55. The method according to claim 54, wherein said inhibitor is octyl gallate.

* * * * *